FORMULATION AND EVALUATION OF CHITOSAN NANOPARTICLES

A dissertation submitted to

THE TAMILNADU Dr.M.G.R MEDICAL UNIVERSITY

CHENNAI- 600 032.

In partial fulfillment of the requirements for the award of Degree of

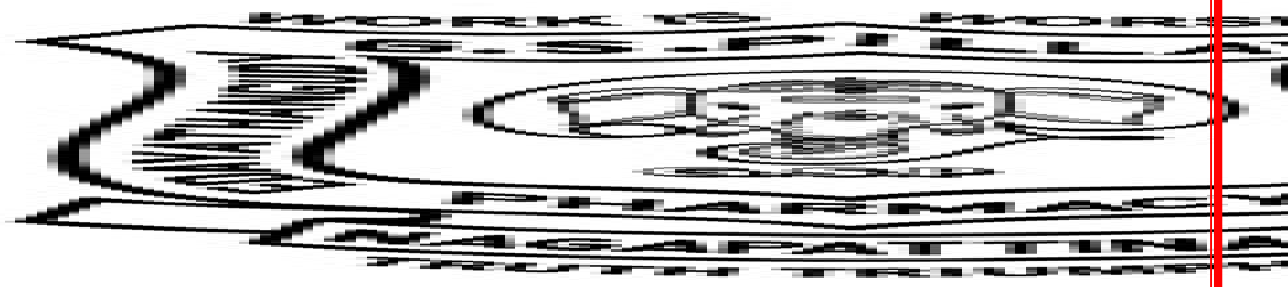
MASTER OF PHARMACY

IN

PHARMACEUTICS

Submitted
By

Reg No: 261211152



DEPARTMENT OF PHARMACEUTICS

EDAYATHANGUDY.G.S PILLAY COLLEGE OF PHARMACY

NAGAPATTINAM-611002

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Under the guidance of

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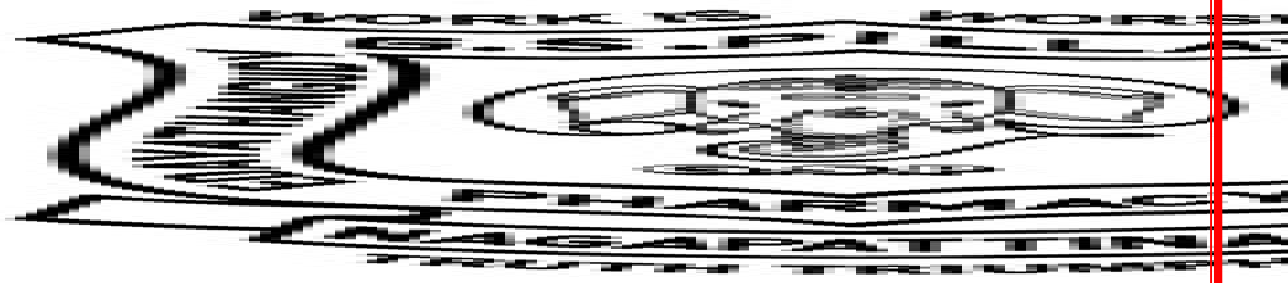

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Professor cum Director,
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CERTIFICATE

This is to certify that the dissertation entitled **"FORMULATION AND EVALUATION OF CHITOSAN NANOPARTICLES"** submitted by **R.Balaji** (Reg No:261211152) in partial fulfillment for the award of degree of Master of Pharmacy to the Tamilnadu Dr. M.G.R Medical University, Chennai is an independent bonafide work of the candidate carried out under my guidance in the Department of Pharmaceutics, Edayathangudy.G.S.Pillay College of Pharmacy during the academic year 2013-2014.

Place: Nagapattinam **Prof.Dr.M.Murugan, M.Pharm., Ph.D.,**

Date:

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Place: Nagapattinam **Prof.Dr.D.BabuAnanth,M.Pharm., Ph.D.,**

Date:

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CONTENTS

INTRODUCTION

Nanoparticulate drug delivery systems (DDS) have attracted a lot of attention because of their size-dependent properties. Among the array of nanoparticles being currently investigated by pharmaceutical scientists, lipid nanoparticles have taken the lead because of obvious advantages of higher degree of biocompatibility and versatility. These systems are commercially viable to formulate pharmaceuticals for topical, oral, pulmonary or parenteral delivery.

Lipid nano formulations can be tailored to meet a wide range of product requirements dictated by disease condition, route of administration and considerations of cost, product stability, toxicity and efficacy. The proven safety and efficacy of lipid-based carriers make them attractive candidates for the formulation of pharmaceuticals, as well as vaccines, diagnostics and nutraceuticals.[1] The most frequent role of lipid-based formulations has traditionally been to improve the solubility of sparingly water soluble drugs especially Biopharmaceutics Classification System (BCS) Classes II & IV drugs. However, the spectrum of applications for lipid-based formulations has widened as the nature and type of active drugs under investigation vary.

Lipid-based formulations may also protect active compounds from biological degradation or transformation that in turn can lead to an enhancement of

drug potency. In addition, lipid-based particulate DDS have been shown to reduce the toxicity of various drugs by changing the bio-distribution of the drug away from sensitive organs. This reduction in toxicity may allow for more drug to be administered and forms the basis for the current success of several marketed lipid-based formulations of amphotericin B (Ambisome®, Abelcet®) and doxorubicin (Doxil®, Myocet®)[1]. Rapid advances in the ability to produce nanoparticles of uniform size, shape, and composition have started a revolution in science. The development of lipid-based drug carriers has attracted increased attention over the last decade.

Lipid nanoparticles (e.g. solid lipid nanoparticles, SLNs) are at the forefront of the rapidly developing field of nanotechnology with several potential applications in drug delivery, clinical medicine and research, as well as in other varied sciences. Due to their size-dependent properties, lipid nanoparticles offer the possibility to develop new therapeutics that could be used for secondary and tertiary level of drug targeting. Hence, lipid nanoparticles hold great promise for reaching the goal of controlled and site specific drug delivery and have attracted wide attention of researchers.

At the turn of the millennium, modifications of SLN, nanostructured lipid carriers (NLC) and lipid drug conjugate (LDC)-nanoparticles were introduced[2,3] in addition to liquid crystal DDS. These carrier systems overcome observed limitations of conventional SLN and more fluid lipid DDS. Compared to

liposomes and emulsions, solid particles possess some advantages, e.g. protection of incorporated active compounds against chemical degradation and more flexibility in modulating the release of the compound. This paper focuses on the different lipid based nano systems, their structure and associated features, stability, production methods, drug incorporation and other issues related to their formulation and use in drug delivery. The following advantages among others could be ascribed to lipid based nanocarriers:

- Ability to control and target drug release.
- Ability to improve stability of pharmaceuticals.
- Ability to encapsulate high drug content (compared to other carrier systems e.g. polymeric nanoparticles).
- The feasibility of carrying both lipophilic and hydrophilic drugs.
- Most of the lipids used are biodegradable, and as such they have excellent biocompatibility, are non-toxic, non-allergenic and non-irritating.
- They can be formulated by water-based technologies and thus can avoid organic solvents.
- They are easy to scale-up and sterilize.
- They are less expensive than polymeric/surfactant based carriers.
- They are easy to validate.

Drug delivery systems

A drug delivery system is defined as a formulation or a device that enables the introduction of a therapeutic substance in the body and improves its efficacy

and safety by controlling the rate, time, and place of release of drugs in the body. The process of drug delivery includes the administration of the therapeutic product, the release of the active ingredients by the product, and the subsequent transport of the active ingredients across the biological membranes to the site of action. DDS interface between the patient and the drug. It may be a formulation of the drug or a device used to deliver the drug[4].

Lipids

The carboxylic acid group of a fatty acid molecule provides a convenient place for linking the fatty acid to an alcohol, via an ester linkage. If the fatty acid becomes attached to an alcohol with a long carbon chain, the resultant substance is called a wax. When glycerol and a fatty acid molecule are combined, the fatty acid portion of the resultant compound is called an acyl group, and the glycerol portion is referred to as a glyceride. A triacylglyceride thus has three fatty acids attached to a single glycerol molecule. Sometimes, this name is shortened to triglyceride. Triglyceride substances are commonly referred to as fats or oils, depending on whether they are solid or liquid at room temperature [5].

A lipid is thus a fatty or waxy organic compound that is readily soluble in non-polar solvents (e.g. ether), but not in polar solvent (e.g. water). Examples of lipids are waxes, oils, sterols, cholesterol, fatsoluble vitamins, monoglycerides, diglycerides, triglycerides (fats), and phospholipids. Fatty acids (including fats) are a subgroup of lipids, hence, it will be inaccurate to consider the terms synonymous.

Lipid drug delivery systems

Lipid-based DDS are an accepted, proven, commercially viable strategy to formulate pharmaceuticals for topical, oral, pulmonary or parenteral delivery. Lipid formulations can be tailored to meet a wide range of product requirements. One of the earliest lipid DDS liposomes have been used to improve drug solubility. Currently, some companies have established manufacturing processes for the preparation of large scale batches of sparingly soluble compounds, often at drug concentrations several orders of magnitude higher than the nominal aqueous solubility because of the introduction of novel lipid-based DDS[1].

Lipid nanoparticulate drug delivery systems

Lipid nanoparticles show interesting nanoscale properties necessary for therapeutic application. Lipid nanoparticles are attractive for medical purposes due to their important and unique features, such as their surface to mass ratio that is much larger than that of other colloidal particles and their ability to bind or adsorb and carry other compounds. Lipid nano formulations produce fine dispersions of poorly water soluble drugs and can reduce the inherent limitations of slow and incomplete dissolution of poorly water soluble drugs (e.g. BCS II & IV drugs), and facilitate formation of solubilised phases from which drug absorption occurs.

In any vehicle mediated delivery system (whether the vehicle is an emulsion, liposome, noisome or other lipidic systems), the rate and mode of drug

release from the system is important in relation to the movement of the delivery system *in vivo*. Lipid particulate DDS abound depending on their architecture and particle size. Due to the large number of administration routes available, these delivery systems perform differently depending on the formulation type and route of administration. Some of the different lipid particulate DDS are shown in Figure 1.

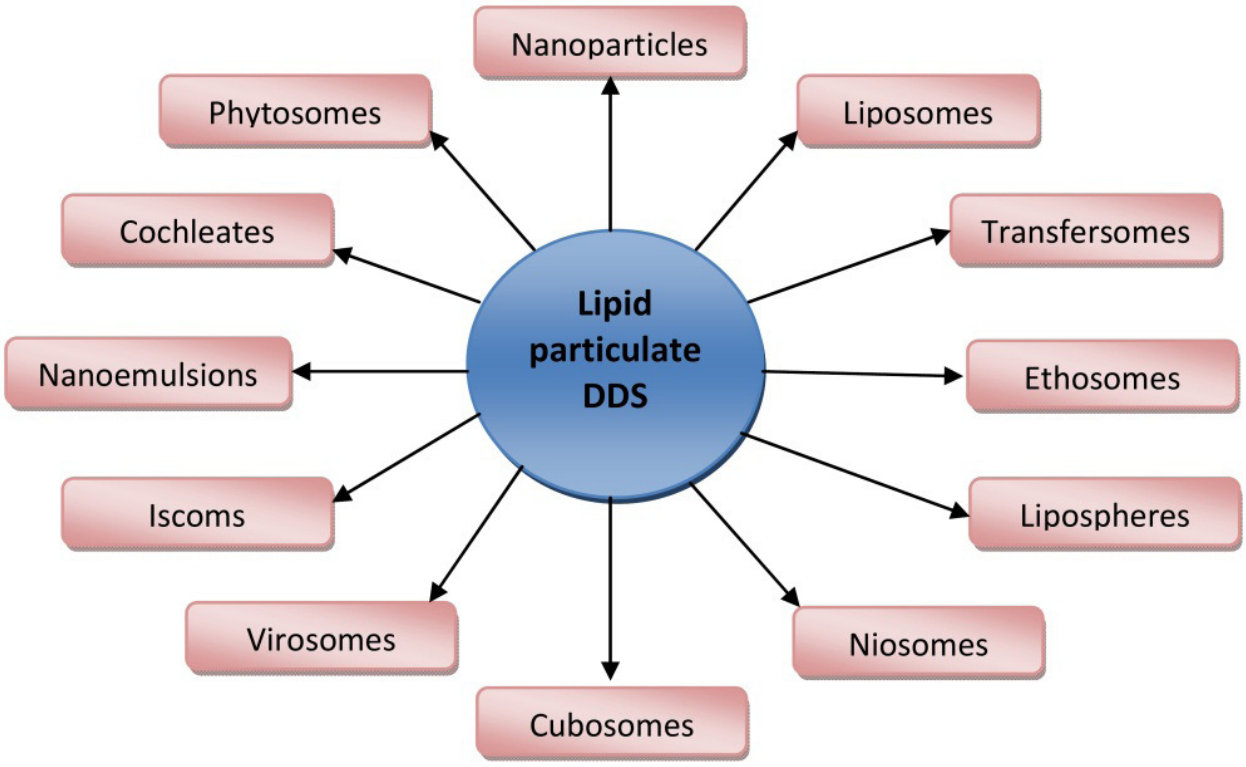


Figure 1. Lipid particulate drug delivery systems

Nanoparticle drug delivery systems are a promising avenue in the field of targeted drug delivery for the treatment of various diseases. The application of this technology improves the bioavailability, physico-chemical stability, and drug loading efficiency. Surface coating, nanoparticle fabrication, fundamental

composition, and drug loading are critical factors that need to be considered during drug design. Examples of this delivery system were successfully employed in the field of brain, cosmetic, dermal, bone fracture, and cancer target deliveries.[6]

To overcome hepatic first-pass metabolism and to enhance bioavailability, intestinal lymphatic transport of drugs can be exploited. Transport of drugs through the intestinal lymphatics via the thoracic lymph duct to the systemic circulation at the junction of the jugular and left subclavian vein, avoids presystemic hepatic metabolism and therefore enhances bioavailability. Highly lipophilic compounds such as long-chain triglycerides reach systemic circulation via the lymphatics.[7]Nanoparticles coated with hydrophobic polymers tend to be easily captured by lymphatic cells in the body. Intraduodenally administered tobramycin-loaded SLN showed sustained release and lymphatic targeting.

Lipid-based drug delivery systems may contain a broad range of oils, surfactants, and co-solvents. They represent one of the most popular approaches to overcome the absorption barriers and to improve the bioavailability of poorly water-soluble drugs. Furthermore, among the factors affecting the bioavailability of the drug from lipid-based formulations are the digestion of lipid, the mean emulsion droplet diameter, the lipophilicity of the drug and the type of lipids. Lipid formulations can reduce the inherent limitation of slow and incomplete dissolution of poorly water-soluble drugs and facilitate formation of solubilized phases from which absorption may occur. The solubilized phases most likely arise

from intral aminal processing after lipid absorption. The co-administration of lipids with drugs can also impact their absorption pathway although most orally administered compounds gain access to the systemic circulation via the portal vein, some highly lipophilic drugs are transported directly to the systemic circulation via intestinal lymphatic's, which improves oral bioavailability of API.[8]

Nanoparticles

Nanoparticles are solid polymeric, submicronic colloidal system range between 5-300nm consisting of macromolecular substances that vary in size 10nm to 1000nm. The drug of interest is dissolved, entrapped adsorbed, attached or encapsulated into the nanoparticles matrix. Depending upon the method of preparation, nanoparticle, nanosphere or nanocapsule can be obtained with different properties and release characteristics for the encapsulated therapeutic agent. Nanosphere are matrix system in which drug is physically and uniformly dispersed throughout, then particles prepared by using different polymers such as polyalkyl cyano-acrylate & poly lactides or they can be solid lipid nanosphere prepared using lipids like dipalmitoyl – phosphatidyl choline. Nanocapsule are ultrafine vesicular system with a diameter less than 1 mcm in which the drug is confined to a cavity surrounded by a unique polymer membrane and having aqueous or oily core containing drug substances. [9]

Nanoparticles holds much interest, because in this range materials can have different and enhanced properties compared with the same materials of a larger

size due to the following two major principle factors. The increased surfaces are of quantum effect. These factors can enhance properties such as reactivity, strength, electrical characteristics & in vivo behavior and a much greater surface area per unit mass compared with the larger particles leading to greater reactivity. The advantages of using nano particles for nanoparticles loaded with drugs, because of their small size can penetrate through small capillaries and are taken up by cells and allow the drug release at right rate and dose at specific sites in the body for a certain time to release the accurate delivery, which enhances the therapeutic effect and reduces the toxicity and side effects. The use of biodegradable materials for nanoparticles preparation allows sustained release within the target site over a period of days or even weeks.

Types of NPS as carrier for drug & diagnostic agents

- Polymeric NPS
- Nanosuspensions and nanocrystals
- Polymeric micelles
- Ceramic NPS
- Liposomes
- Fullerenes and dendrimers
- SLNP (Solid lipid nanoparticles)
- Magnetic nanoparticles
- Nanoshells coated with gold
- Nanomers and carbon nanotubes[10]

Solid lipid nanoparticles (SLN)

SLN are particulates structurally related to polymeric nanoparticles. However, in contrast to polymeric systems, SLN can be composed of

biocompatible lipids that are physiologically well tolerated when administered *in vivo* and may also be prepared without organic solvents. The lipid matrices can be composed of fats or waxes (homolipids) that provide protection to the incorporated bioactive from chemical and physical degradation, in addition to modification of drug release profile. Typical formulations utilize lipids such as paraffin wax or biodegradable glycerides (e.g. Compritol 888 ATO) as the structural base of the particle[11].

SLN were developed in the 1990s as an alternative carrier system to the existing traditional carriers, such as emulsions, liposomes and polymeric nanoparticles. SLN are prepared either with physiological lipids or lipids with generally regarded as safe (GRAS) status. Under optimized conditions they can incorporate lipophilic or hydrophilic drugs and seem to fulfil the requirements for an optimum particulate carrier system[12]. SLN have a potential wide application spectrum- parenteral administration and brain delivery, ocular delivery, rectal delivery, oral delivery, topical delivery and vaccine delivery systems etc., in addition to improved bioavailability, protection of sensitive drug molecules from the outer environment and even controlled release characteristics. Common disadvantages of SLN are particle growth, unpredictable gelation tendency, unexpected dynamics of polymorphic transitions and inherent low incorporation rate due to the crystalline structure of the solid lipid[13].

LITERATURE REVIEW

1. **Muller RH et al.,** studied the solid Lipid nanoparticles (SLN) and nano structured lipid carriers (NLC) in cosmetic and dermatological preparations and reported the involvement and improvement of the delivery of cosmetic preparations when it's delivered in the form of solid lipid nanoparticles.

2. **Muller RH et al.,** studied the medicament vehicle for the controlled administration of an active agent, produced from lipid matrix-medicament conjugates. They reported the delivery of medicament conjugate and its advantage when it was delivered in the lipid matrix.

3. **Goswami S et al.,** reviewed lovastatin and its production. The biotechnological production of lovastatin and its simplicity in production was reviewed. The lipid lowering drug lovastatin acting on the HMG-Co A and its production was done and isolation and purification was discussed. The review also deals with the structure, properties, biosynthetic pathway, submerged fermentation, applications and side effects of lovastatin.

4. **Jawahar N et al.,** solid lipid nanoparticles for oral delivery of poorly soluble drugs. They reported the advantages and disadvantages of solid lipid nanoparticles. Various methods of preparation of nanoparticles and their evaluation were also reported. They also reported the applications of nanoparticles in pharmaceutical field. They discussed the physico-chemical properties of solid lipid nanoparticles, Lymphatic mechanism, production methods, *in-vivo* fate of lipids and potential therapeutic applications.

5. **Karthik neduri et al.,** studied the different techniques to enhance the dissolution rate of lovastatin: formulation and evaluation. They studied enhancement of the dissolution profile, absorption efficiency and bioavailability of water insoluble drugs like Lovastatin by using various techniques. Lovastatin is a poorly soluble, highly permeable drug and the rate of its oral absorption is often controlled by the dissolution rate in the gastrointestinal. They made an attempt to enhance the *in vitro* dissolution profile of Lovastatin by using various methods like solid dispersions, superdisintegrants and sublimation with respective to conventional drug.

6. **Gande Suresh et al.,** studied whether the bioavailability of lovastatin could be improved by administering lovastatin solid lipid nanoparticles (SLN) duodenally to rats. Lovastatin SLN were developed using triglycerides by hot homogenization followed by ultrasonication. Particle size and zeta potential were measured by photon correlation spectroscopy. Bioavailability studies were conducted in male Wistar rats after intraduodenal administration of lovastatin suspension and SLN. Stable lovastatin SLN had mean size range of 60 to 119 nm and a zeta potential range of –16 to –21 mV. More than 99% of the lovastatin was entrapped in the SLN. Lovastatin was dispersed in an amorphous state, and triglycerides were in β1 form in the SLN. In vitro stability studies showed the slow release and stability of lovastatin SLN. The relative bioavailabilities of

lovastatin and lovastatin hydroxy acid of SLN were increased by ~173% and 324%, respectively, compared with the reference lovastatin suspension.

7. **Pragati et al.,** reviewed the solid lipid nanoparticles and reported the advantages of solid lipid nanoparticles delivery system. The solid lipid nanoparticles are bioacceptable and biocompatible in nature since less toxic. The review mainly focused on preparation, advantages, characterization and special features.

8. **Muller RH et al.,** reviewed the solid lipid nanoparticles and reported the importance of the SLN in drug delivery. They have reported the ability of the SLN to deliver the drug in controlled manner.

9. **Seenivasan A et al.,** reported the nano drug delivery system, the latest technology employed in various medicinal applications. This technology can be adapted to the conventional drug administration due to its site-specific targeting phenomena. The oral lipophilic drug administration has its drawbacks due to poor solubility and bioavailability. Lipid-based carrier systems are now widely popular due to improved efficiency, especially for lovastatin delivery. Lovastatin is an important drug which arrests the rate-limiting step of the cholesterol cascade. This drug has short half-lives, poor oral-administered bioavailability, poor solubility, and is rapidly metabolizable. Based on the composition, the drug delivery carriers are

classified into solid lipid nanoparticles (SLNs), lipid emulsions (LEs), and nanostructured lipid carriers (NLCs). Among them, NLCs are a smarter generation of drug delivery carriers for lovastatin. The selection of various lipid systems and their formulation are discussed. Moreover, the characterization of these carrier systems to achieve the optimal characteristic features is discussed in a concise manner.

10. **Eldem et al.,** studied the importance of polymorphism occurring in solid dosage forms causing instability, the polymorphic behavior of spray-dried and -congealed lipid micropellets was examined by differential scanning calorimetry and scanning electron microscopy. The results showed that both of the spraying processes exert an important effect on their polymorphic and crystallization properties. In spray-drying, due to the rapid solvent evaporation, the obtained lipid micropellets possess an unstable polymorphic form. This unstable form transforms gradually toward a stable form by storage at elevated temperatures. The same modifications were observed with spray-congealed lipid micropellets. The type of glyceride (composition, chain length), solvent and drugs (estradiol cypionate, medroxyprogesterone acetate) and, further, the presence of a stabilizing agent such as lecithin affect the polymorphic transition and its rate.

11. **Patricia Severino et al.,** reported the Lipids and lipid nanoparticles. These have been exploited for many features in the field of pharmaceutical technology. Lipids usually enhance drug absorption in the gastrointestinal tract (GIT), and when formulated as nanoparticles, these molecules improve mucosal adhesion due to small particle size and increasing their GIT residence time. In addition, lipid nanoparticles may also protect the loaded drugs from chemical and enzymatic degradation and gradually release drug molecules from the lipid matrix into blood, resulting in improved therapeutic profiles compared to free drug. Therefore, due to their physiological and biodegradable properties, lipid molecules may decrease adverse side effects and chronic toxicity of the drug-delivery systems when compared to other of polymeric nature. They highlighted the importance of lipid nanoparticles to modify the release profile and the pharmacokinetic parameters of drugs when administrated through oral route.

12. **Wolfgang Mehnert et al.,** studied the solid lipid nanoparticles and its production, characterization and applications in pharmaceuticl field. The reported the various methods for production of nanoparticles.

13. **Mehnert W et al.,** reported the solid lipid nanoparticles production, characterization. They also reported the applications of solid lipid nanoparticles in various therapies.

14. **Mukherjee et al.,** studied and reported the solid lipid nanoparticles. The results concluded the solid lipid nanoparticles are used as modern formulation approach in drug delivery system.

15. **Wissing et al.,** reported the solid lipid nanoparticles and their application in the pharmaceutical field. They also studied the use of SLN when its delivered in parenteral route.

16. **Cortesi et al.,** evaluated the production of lipospheres. The lipospheres formulated by using the lipid carriers and reported their use to deliver deliver bioactive compounds.

17. **Rudolph et al.,** studied the application of novel solid lipid nanoparticles (SLN)-gene vector formulations. They formulated the diametric HIV-1 VAT-peptide and evaluated them *in vitro and in vivo*.

18. **Arunkumar. N et al.,** attempted to improve the solubility and dissolution characteristics of a poorly soluble drug using nanosuspension technology. Nanoparticles were characterized in terms of size and morphological characteristics. Saturation solubility and dissolution characteristics were investigated and compared to the commercial drug. Crystallinity of the drug was also evaluated by performing thermal gravimetric analysis (TGA), differential scanning calorimetry (DSC) and powder X-ray diffraction

(PXRD) to denote eventual transformation to amorphous state during the homogenization process. The study shown that the crystalline state of the drug is reduced following particle size reduction and the dissolution rates of amorphous atorvastatin calcium nanoparticles were highly increased in comparison with commercial drug by the enhancement of intrinsic dissolution rate and the reduction of particle size, resulting in an increased specific surface area.

19. **Panakanti Gayatri et al.,** studied the formulation of Atorvastatin loaded solid lipid nanoparticles by hot homogenization fallowed by ultrasonication technique, and optimization of formulation and process parameters to formulate preferred SLN dispersions. The effects of composition of lipid materials, surfactant mixture and sonication time on particle size, PDI, zeta potential, drug entrapment efficiency, and in vitro drug release behavior were investigated. The mean particles size, PDI, zeta potential and entrapment efficiency of optimized formulation (A5) was found to be 50.0± 6.12 nm, 0.08±0.011,10.40± 4.68mV, 88.7± 6.08 % respectively. To characterize the state of drug and lipid modification in ATRS loaded solid lipid nanoparticles, differential scanning calorimetry analysis was performed. Shape and surface morphology was determined by Transmission Electron Microscopy (TEM) which revealed fairly spherical shape of nanoparticles. The in-vitro drug release study demonstrated that

ATRS-SLN formulation (A5) possessed controlled drug release over a period of 24 hrs than dispersion of pure drug.

20. **Seenivasan et al.,** studied the oral lipophilic drug administration and its drawbacks due to poor solubility and bioavailability. Lovastatin is an important drug which arrests the rate-limiting step of the cholesterol cascade. This drug has short half-lives, poor oral-administered bioavailability, poor solubility, and is rapidly metabolizable. Based on the composition, the drug delivery carriers are classified into solid lipid nanoparticles (SLNs), lipid emulsions (LEs), and nanostructured lipid carriers (NLCs). Among them, NLCs are a smarter generation of drug delivery carriers for lovastatin. The characterization of these carrier systems to achieve the optimal characteristic features is discussed in a concise manner.

21. **Radha et al.,** reviewed the production of lovastatin, while other statins like rosuvastatin, simvastatin, pravastatin, fluvastatin are synthesized from lovastatin and mevastatin. Other than reduction of cholesterol, lovastatin is shown to provide various medicinal properties like anti-cancer, bone maturation, multiple sclerosis. This review discussed production of lovastatin and different fermentation techniques. This review also helps in understating the inhibition of HMG-CoA reducatse and various medicinal properties of lovastatin.

22. **Raja Rajeswari et al.,** attempted to improve the solubility and dissolution rate using solid dispersion of a poorly soluble drug Lovastatin by using Soluplus as carrier material to enhance the solubility as well as dissolution rate. Six different formulations were prepared using hot melt extrusion technique in different ratios i.e., 1:1, 1:2, 1:3, 1:5, 1:7, and 1:9 and were further characterized by FTIR, DSC, and SEM analysis. SEM studies showed the surface morphology of the solid dispersion. All the formulations showed a marked increase in drug release with the increase in the concentration of soluplus when tested for their in vitro studies. Formulation F6 showed the best release with a cumulative release of 99% in 50 mins when compared to the pure drug, Physical mixture and marketed formulation. Hence, soluplus look to be a promising carrier to improve the solubility of poorly soluble drugs.

23. **Shalini Asthana et al.,** A developed RP-HPLC method for the purpose of analysis of antihypertensive: nifedipine (NF), antidiabetic: nateglinide (NG) and hypolipidemic: lovastatin (LT) drugs simultaneously in cardiovascular polypill based synthetic ternary mixture. The validated method was successfully applied to the analysis of synthetic mixture of tablets of three drugs; the percentage recoveries obtained were 100.23% for NF, 100.35% for NG and 100.93% for LT.

24. **Karthik neduri et al.,** studied to enhance the dissolution profile, absorption efficiency and bioavailability of water insoluble drugs like Lovastatin by using various techniques. Lovastatin is a poorly soluble, highly permeable drug and the rate of its oral absorption is often controlled by the dissolution rate in the gastrointestinal tractthe results suggest that there was satisfactory dissolution enhancement from all three methods and could potentially lead to improvement in bioavailability of oral lovastatin products, but superdisintegrant method was preferred due to its simplicity, low cost and industrial feasibility.

25. **Manjil Patel et al.,** improved the solubility of poorly water soluble drug lovastatin (LS) by solid dispersion (SD) techniques using modified locust bean gum (MLBG) as a carrier. The locust bean gum (LBG) was modified by heating and there observed irreversible decrease in viscosity, whereas swelling property remains unaffected. Dissolution study revealed that the modified solvent evaporation is most convenient and effective method for solubility enhancement of poorly water soluble drug LS, among various methods of preparation of SD. The prepared SDs was characterized by differential scanning calorimetry, scanning electron microscopy, and X-ray diffraction study. In vivo study was performed by measuring 3-hydroxy-3-methyl-glutaryl-coenzyme A (HMG Co-A) reductase inhibition activity. Significant reduction in HMG Co-A reductase activity was observed in case of solid dispersions of LS than plain LS.

26. **Xiao Gu.,** investigated a non-protein nanostructured lipid carrier (NLC) resembling high-density lipoprotein (HDL) could deliver a hydrophobic anti-atherogenic drug, lovastatin, to foam cells. Lovastatin-loaded NLC (LT-NLC) was prepared by a nanoprecipitation/solvent diffusion method. The LT-NLC-apoprotein (LT-NLC-apo) was prepared by incubating LT-NLC with native HDL. The results indicated that non-protein NLC resembling HDL could be a useful tool to deliver lipophilic anti-atherogenic drugs to foam cells, and that uptake could be enhanced by the VLDL receptor pathway.

27. **Faghfouri et al.,** reported the optimum condition for solid lipid nanoparticles preparation. The method used to obtain nanoparticles with the size of about 220 nm and PDI of about 0.25. The optimized set of method variables were determined and validated for the new SLN preparation method.

28. **Raksha L.Mhetre.,** developed developed controlled release SLNs by incorporating the drug in to lipid nanocarriers. The nanosize of particles and lipid used for formulation would help to improve solubility of drug as well as control drug release over prolonged period. SLNs were prepared by High shear homogenization (HSH) technique using stearic acid as solid

lipid core, Tween 80 and Poloxamer 188 as stabilizers. Palatability of nanodispersion was improved using sweetener and flavors.

29. **Makarand Gambhire et al.,** attempted to improve oral bioavailability of simvastatin by incorporating in solid lipid nanoparticles (SLN). The purpose of thie research was to study whether the oral bioavailability of simvastatin could be improved by administering simvastatin loaded SLNs. Simvastatin SLNs were developed using compritol 888 ATO by pre-emulsion followed by ultrasonication and characterized by photon correlation spectroscopy, DSC and XRD. Bioavailability studies were conducted in albino rats after oral administration of simvastatin suspension and SLN. The obtained results indicated SLNs as potential carriers for improving the bioavailability of poorly bioavailable drugs such as simvastatin by minimizing first pass metabolism.

AIM OF WORK

Presently, many research groups are trying to explore the possibility of using solid lipid nanoparticles (SLN) as drug carriers. The concept of SLN was first investigated a decade ago to unravel problems associated with other colloidal drug delivery systems, such as instability and non biodegradability. SLN are widely used to improve bioavailability and to achieve sustained release.

To overcome the drawbacks associated to the traditional colloidal systems such as emulsions, Liposomes, and polymeric nanoparticles solid lipid nanoparticles (SLN) have been developed.

- SLN are biocompatible and biodegradable and have been used for controlled drug delivery and specific targeting.
- These colloidal carriers consist of a lipid matrix that should be solid at both room and body temperatures, having a mean particle size between 50 nm and 1000nm.which are dispersed in water or aqueous surfactant solution.
- They are made up of solid hydrophobic core having a monolayer of phospholipid coating. Solid core contains the drug dispersed or dissolved in lipid matrix.
- They have potential to carry lipophilic or hydrophilic drugs.

Lovastatin is a poorly soluble drug with a shorter half life of 1.1-1.7 h and less than 5% bioavailability so an attempt was to formulate solid lipid nanoparticles of lovastatin. Due to the following advantages of lipid based drug delivery systems like solid lipid nano plarticles,

- Lipid-based drug delivery systems may contain a broad range of oils, surfactants, and co-solvents.
- They represent one of the most popular approaches to overcome the absorption barriers and to improve the bioavailability of poorly water-soluble drugs.
- Furthermore, among the factors affecting the bioavailability of the drug from lipid-based formulations are the digestion of lipid, the

mean emulsion droplet diameter, the lipophilicity of the drug and the type of lipids.

- Lipid formulations can reduce the inherent limitation of slow and incomplete dissolution of poorly water-soluble drugs and facilitate formation of solubilized phases from which absorption may occur.
- The solubilized phases most likely arise from intral aminal processing after lipid absorption.
- The co-administration of lipids with drugs can also impact their absorption pathway although most orally administered compounds gain access to the systemic circulation via the portal vein, some highly lipophilic drugs are transported directly to the systemic circulation via intestinal lymphatic's, which improves oral bioavailability of poorly water soluble API like lovastatin.[8]

The aim of the current work is to formulate and evaluate the solid lipid nanoparticles of lovastatin to deliver sustained drug release. Due to the lipid nature of solid lipid nanoparticles of lovastatin the problems associated with conventional formulations like poor solubility and less bioavailability can be improved in a reasonable manner.

Statins are compounds of natural origin that are biosynthesized as secondary metabolites of several filamentous fungi and act as competitive inhibitors of HMG-CoA reductase. They are bulky and literally get "stuck" in the active site. This prevents the enzyme from binding with its substrate, HMG-CoA. Today, there are two classes of statins:

- **Natural Statins:** Lovastatin (Mevacor), Compactin, Pravastatin (Pravachol), Simvastatin (Zocor).
- **Synthetic Statins:** Atorvastatin (Lipitor), Fluvastatin (Lescol). The most common statins are atorvastatin (Lipitor), fluvastatin (Lescol), lovastatin (Mevacor, Altocor), pravastatin (Pravachol), simvastatin (Zocor), and rosuvastatin (Crestor).

Lovastatin, a specific and potent competitive inhibitor of 3-hydroxy- 3-methyl glutaryl coenzyme A (HMG-CoA) is a powerful serum cholesterol-lowering drug in humans and other species. It is formerly called as mevinolin; monacolin K, and mevacor and it is a fungal secondary metabolite which inhibits HMG-CoA reductase, the first committed enzyme of cholesterol biosynthesis. The endogenous synthesis of cholesterol is carried out by the mevalonate pathway, in which the rate limiting reaction is the conversion of (S) HMG-CoA to (R) mevalonate, catalyzed by HMG-CoA reductase.

The history of statin began in 1987 when the lovastatin received Food and Drug Administration (FDA) approval in the USA. Lovastatin have revolutionized the treatment of hypercholesterolemia and it is proven that lovastatin is also therapeutically and preventatively effective in the treatment of major kind of diseases like atherosclerosis, sepsis, peripheral arterial disease, peripheral vascular disease, cerebro vascular disease, ischemic disease, and bone fracture.[11]

Lovastatin lowers cholesterol levels through reversible and competitive inhibition of 3-hydroxy- 3-methylglutaryl coenzyme A reductase, an enzyme

involved in the biosynthesis of cholesterol. It exhibits poor oral bioavailability because of rapid metabolism in the gut and liver. It's a poorly soluble drug with a shorter half life of 1.1-1.7 h and less than 5% bioavailability.[12] Cytochrome P450 3A4 metabolizes the lactone form of lovastatin into hydroxy acid and its metabolites.[11]

Lovastatin (whose water solubility is 0.4 × 10−3 mg/mL) is considered to be a reasonable substrate for intestinal lymphatic transport because of its high log P value (4.3) and good solubility in oils (38 and 42 mg/mL in carbitol and propylene glycol monocaprylate, respectively).

The solid lipid nanoparticles of lovastatin would be an alternate to conventional formulations by providing sustained drug release and improved bioavailability due to improved solubility.

PLAN OF WORK

1. Determination of max of lovastatin
2. Calibration curve for the drug in phosphate buffer pH 7.4
3. Drug polymer interaction study by using FTIR
4. Formulation of solid lipid nanoparticles of lovastatin by hot homogenization technique by using different concentrations of surfactant.
5. Evaluation of particle size
6. Determination of percentage drug entrapment efficiency
7. Evaluation of *in vitro* drug release.
8. Comparisons of *In –vitro* release pattern of optimized solid lipid nanoparticles of lovastatin with lovastatin pure drug solution.
9. Stability studies of optimized solid lipid nanoparticles of lovastatin.

MATERIALS AND EQUIPMENTS

MATERIALS USED

1. Drug- Lovastatin - Cipla laboratories
2. Stearic acid - Hospira laboratories
3. Poloxamer - Hospira laboratories
4. Tween 80 - Ultra Chem laboratories
5. Di-sodium hydrogen phosphate - Vin biotech systems
6. Sodium hydroxide - Nice chemicals

EQUIPMENTS USED

1. Homogenizer - Bombay India ltd.

2. Malvern zeta sizer - SM, UK.
3. Electronic Balance - A&D Company, Japan
4. Magnetic Stirrer - MC Dalal & co
5. UV Visible Spectrophotometer - UV Pharma spec 1700, Shimadzu
6. FTIR Spectrophotometer - Perkin Elmer, Model - 78625.
7. Environmental chamber - Inlab equipments (Madras pvt ltd)

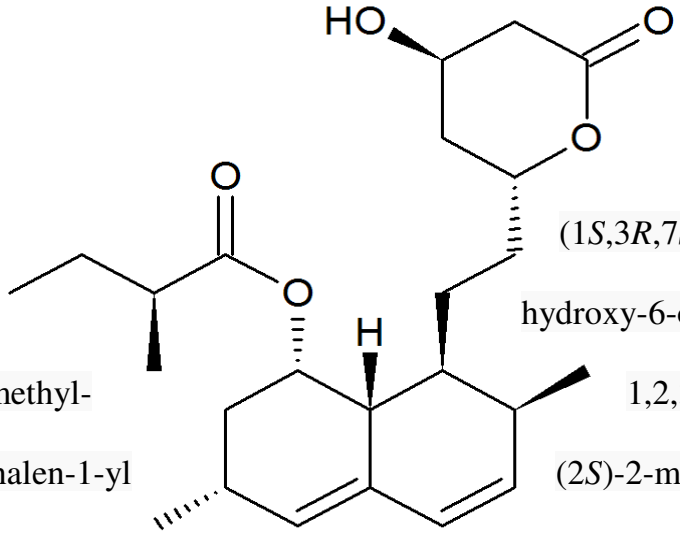
DRUG PROFILE

Lovastatin

Statins are compounds of natural origin that are biosynthesized as secondary metabolites of several filamentous fungi and act as competitive inhibitors of HMG-CoA reductase. They are bulky and literally get “stuck” in the active site.

Structural formula

IUPAC Name



(1*S*,3*R*,7*S*,8*S*,8a*R*)-8-{2-[(2*R*,4*R*)-4-hydroxy-6-oxooxan-2-yl]ethyl}-3,7-dimethyl-1,2,3,7,8,8a-hexahydronaphthalen-1-yl (2*S*)-2-methylbutanoate

Properties:

Water solubility	0.0004 mg/mL
logP	4.11
logs	-4.2
pKa (strongest acidic)	14.91
pKa (strongest basic)	-2.8
Physiological charge	0
Hydrogen acceptor count	3
Hydrogen donor count	1
Polar surface area	72.83
Polarizability	46.11

Melting point	174.5 °C

Dosing

The dose range for lovastatin is 10-80 mg daily given preferably in the evening when it may be most effective. The usual starting dose is 20 mg once daily, and the maximum dose is 80 mg daily. Blood cholesterol determinations are performed at regular intervals during treatment so that adjustments in dosage can be made.[39]

Protein binding

Lovastatin and its β-hydroxyacid metabolites are highly protein bound (>95%).

Metabolism

Lovastatin is hepatically metabolized in which the major active metabolites are the β-hydroxyacid of lovastatin, the 6'-hydroxy derivative, and two additional metabolites (CYP3A substrate).

Route of elimination

Lovastatin undergoes extensive first-pass extraction in the liver, its primary site of action, with subsequent excretion of drug equivalents in the bile. 83% of the orally administered dose is excreted in bile and 10% is excreted in urine.

Bioavailability

<5%

Half-life

1.1-1.7 hours

Clearance

Not Available

Pharmacodynamics

The primary cause of cardiovascular disease is atherosclerotic plaque formation. Sustained elevations of cholesterol in the blood increase the risk of cardiovascular disease. Lovastatin lowers hepatic cholesterol synthesis by competitively inhibiting HMG-CoA reductase, the enzyme that catalyzes the rate-limiting step in the cholesterol biosynthesis pathway via the mevalonic acid pathway. Decreased hepatic cholesterol levels causes increased uptake of low density lipoprotein (LDL) cholesterol and reduces cholesterol levels in the circulation. At therapeutic doses, lovastatin decreases serum LDL cholesterol by 29-32%, increases high density lipoprotein (HDL) cholesterol by 4.6-7.3%, and decrease triglyceride levels by 2-12%. HDL cholesterol is thought to confer protective effects against CV disease, whereas high LDL and triglyceride levels are associated with higher risk of disease. [40]

Mechanism of action

Lovastatin is structurally similar to the HMG, a substituent of the endogenous substrate of HMG-CoA reductase. Lovastatin is a prodrug that is activated *in vivo* via hydrolysis of the lactone ring to form the β-hydroxyacid. The hydrolyzed lactone ring mimics the tetrahedral intermediate produced by the reductase allowing the agent to bind to HMG-CoA reductase with 20,000 times greater affinity than its natural substrate. The bicyclic portion of lovastatin binds to the coenzyme A portion of the active site.

Absorption

Studies suggest that <5% of the oral dose reaches the general circulation as active inhibitors. Time to peak serum concentration is 2-4 hours. Lovastatin undergoes extensive first-pass metabolism so the availability of the drug in the system is low and variable.

Volume of distribution

Lovastatin is able to cross the blood-brain-barrier and placenta.

Side effects

Lovastatin is usually well tolerated. As with all statin drugs, it can rarely cause myopathy or rhabdomyolysis. This can be life-threatening if not recognised and treated in time, so any unexplained muscle pain or weakness whilst on lovastatin should be promptly mentioned to the prescribing doctor. Other uncommon side effects that should be promptly mentioned to either the prescribing doctor or an emergency medical service include:

- Muscle pain, tenderness, or weakness

- Lack of energy
- Weakness
- Fever
- Dark colored urine
- Jaundice: yellowing of the skin or eyes
- Pain in the upper right part of the stomach
- Nausea
- Unusual bleeding or bruising
- Loss of appetite
- Flu-like symptoms
- Rash
- Hives
- Itching
- Difficulty breathing or swallowing
- Swelling of the face, throat, tongue, lips, eyes, hands, feet, ankles, or lower legs

- Hoarseness

Indication

For management as an adjunct to diet to reduce elevated total-C, LDL-C, apo B, and TG levels in patients with primary hypercholesterolemia and mixed dyslipidemia. It is also used for primary prevention of coronary heart disease and to slow progression of coronary atherosclerosis in patients with coronary heart disease.

Uses

Lovastatin is used along with a proper diet to help lower "bad"cholesterol and fats (such as LDL, triglycerides) and raise "good" cholesterol (HDL) in the blood. It belongs to a group of drugs known as "statins." It works by reducing the amount of cholesterol made by the liver. Lowering "bad" cholesterol and triglycerides and raising "good" cholesterol decreases the risk of heart disease and helps prevent strokes and heart attacks.In addition to eating a proper diet (such as a low-cholesterol/low-fat diet), other lifestyle changes that may help this medication work better include exercising, losing weight if overweight, and stopping smoking. Consult your doctor for more details.

Storage

Immediate release tablets should be stored between 5-30^0 C (41-86 F). Extended release tablets should be stored at room temperature, 20-25^0 C (68-77 F).

[41]

EXCIPIENTS PROFILE

Poloxamer

Nonproprietary Names

Poloxamers

Synonyms

Lutrol; Monolan; Pluronic; poloxalkol; poloxamera; polyethylene–propylene glycol copolymer; polyoxyethylene–polyoxypropylene copolymer; Supronic; Synperonic.

Chemical Name and CAS Registry Number

a-Hydro-o-hydroxypoly(oxyethylene)poly(oxypropylene) poly- (oxyethylene) block copolymer [9003-11-6]

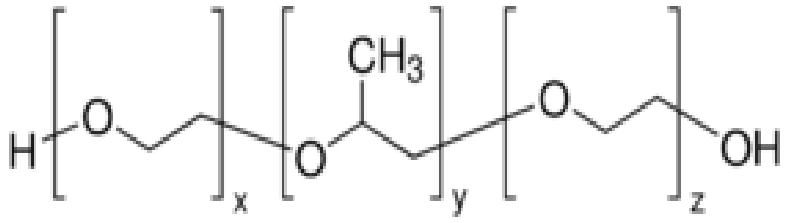
Empirical Formula and Molecular Weight

The poloxamer polyols are a series of closely related block copolymers of ethylene oxide and propylene oxide conforming to the general formula HO(C2H4O)a(C3H6O)b(C2H4O)aH.

Functional Category

It is used as dispersing agent, emulsifying agent, solubilizing agent, tablet lubricant and wetting agent.

Structural Formula



Applications in Pharmaceutical Formulation or Technology

Poloxamers are nonionic polyoxyethylene–polyoxypropylene copolymers used primarily in pharmaceutical formulations as emulsifying or solubilizing agents. The polyoxyethylene segment is hydrophilic while the polyoxypropylene segment is hydrophobic. All of the poloxamers are chemically similar in composition, differing only in the relative amounts of propylene and ethylene oxides added during manufacture. Their physical and surface-active properties vary over a wide range and a number of different types are commercially available. Poloxamers are used as emulsifying agents in intravenous fat emulsions,

and as solubilizing and stabilizing agents to maintain the clarity of elixirs and syrups.

Poloxamers may also be used as wetting agents; in ointments, suppository bases, and gels; and as tablet binders and coatings. Poloxamer 188 has also been used as an emulsifying agent for fluorocarbons used as artificial blood substitutes, and in the preparation of solid-dispersion systems. More recently, poloxamers have found use in drug-delivery systems.

Therapeutically, poloxamer 188 is administered orally as a wetting agent and stool lubricant in the treatment of constipation; it is usually used in combination with a laxative such as danthron. Poloxamers may also be used therapeutically as wetting agents in eye-drop formulations, in the treatment of kidney stones, and as skin-wound cleansers. Poloxamer 338 and 407 are used in solutions for contact lens care.

S.No	**Uses**	**Concentration (%)**
1.	Fat emulsifier	0.3

2.	Flavor solubilizer	0.3
3.	Fluorocarbon emulsifier	2.5
4.	Gelling agent	15–50
5.	Spreading agent	1
6.	Stabilizing agent	1–5
7.	Suppository base	4–6 or 90
8.	Tablet coating	10
9.	Tablet excipient	5–10
10.	Wetting agent	0.01–5

Description

Poloxamers generally occur as white, waxy, free-flowing prilled granules, or as cast solids. They are practically odorless and tasteless. At room temperature, poloxamer 124 occurs as a colorless liquid.

Typical Properties

Acidity/alkalinity	pH = 5.0–7.4 for a 2.5% w/v aqueous solution.
Cloud point	>100^{0}C for a 1% w/v aqueous solution, and a 10% aqueous solution of poloxamer 188.
Density	1.06 g/cm3 at 25^{0}C
Flash point	260^{0}C
Flowability	Solid poloxamers are free flowing.
HLB value	0.5–30; 29 for poloxamer 188.

Melting point

It is 52–57^{0}C for poloxamer 188.

Moisture content

Poloxamers generally contain less than 0.5% w/w water and are hygroscopic only at relative humidity greater than 80%.

Solubility

Solubility varies according to the poloxamer type.

Surface tension

19.8mN/m (19.8 dynes/cm) for a 0.1% w/v aqueous poloxamer 188 solution at 25^0C;

24.0mN/m (24.0 dynes/cm) for a 0.01% w/v aqueous poloxamer 188 solution at 25^0C;

26.0mN/m (26.0 dynes/cm) for a 0.001% w/v aqueous poloxamer solution at 25^0C.

Viscosity (dynamic)

1000 mPa s (1000 cP) as a melt at 77^0C for poloxamer 188.

Stability and Storage Conditions

Poloxamers are stable materials. Aqueous solutions are stable in the presence of acids, alkalis, and metal ions. However, aqueous solutions support

mold growth. The bulk material should be stored in a well-closed container in a cool, dry place.

Incompatibilities

Depending on the relative concentrations, poloxamer 188 is incompatible with phenols and parabens. [42, 43]

Polysorbate 80

Synonyms

Polysorbate 80, Polyoxyethylene Sorbitan Monooleate, Monitan, Ethoxylated Sorbitan Monooleate, Sorbital O 20, Sorlate, Polyethylene glycol sorbitan monooleate

Product information

It's a nonionic surfactant and emulsifier derived from polyethoxylated sorbitan and oleic acid, and is often used in foods. Polysorbate 80 is a viscous, water-soluble yellow liquid. The hydrophilic groups in this compound are polyethers also known as polyoxyethylene groups which are polymers ofethylene oxide. In the nomenclature of polysorbates, the numeric designation following polysorbate refers to the lipophilic group, in this case the oleic acid (see polysorbate for more detail). Polysorbate 80 is often used in food and other products as an emulsifier.

Molecular Formula

$C_{32}H_{60}O_{10}$

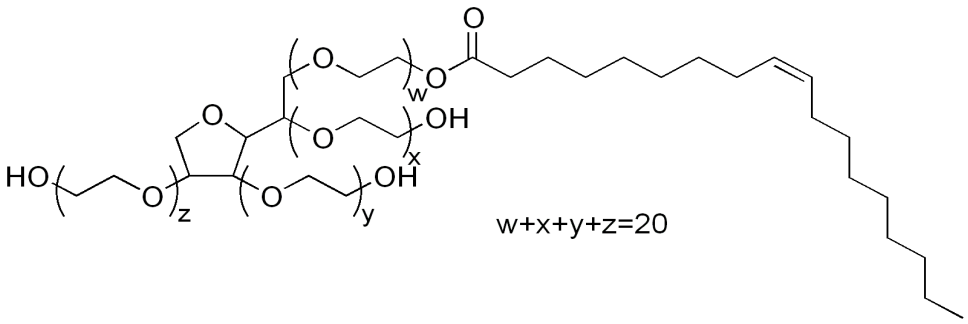
Molecular Weight

604.8128

IUPAC Name:

2-[2-[3,4-bis(2-hydroxyethoxy)oxolan-2-yl]-2-(2-hydroxyethoxy)ethoxy]ethyl(E)-octadec-9-enoate

Structural Formula



Product Description

Tween 80 is a polyethylene sorbitol ester, non ionic surfactant and an emulsifier derived from polyethoxylated sorbitan and oleic acid.

Molar mass 1310 g/mol

Appearance	Amber colored viscous liquid
Density	1.06–1.09 g/mL, oily liquid
Boiling point	> 100°C
Solubility in water	Very soluble
Solubility in other solvents	Soluble in ethanol, cottonseed oil, corn oil, ethyl acetate, methanol, toluene

Applications

Tween 80 has been widely used in biochemical applications including: solubilizing proteins, isolating nuclei from cells in culture, growing of tubercule bacilli, and emulsifying and dispersing substances in medicinal and food products. It has little or no activity as an anti-bacterial agent. It has been shown to have an adverse effect on the antibacterial effect of methyl paraben and related compounds. [44,45]

Stearic acid

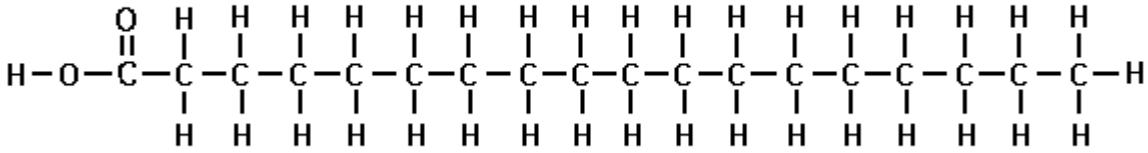
Synonyms

Acidum stearicum, cetylacetic acid, Crodacid, Cristal G, Cristal S, Dervacid, E570, Edenor, Emersol, Extra AS, Extra P, Extra S, Extra ST, 1-heptadecanecarboxylic acid, Hystrene, Industrene, Kortacid 1895, Pearl Steric, Pristerene, stereophanic acid and Tegostearic.

Empirical Formula and Molecular Weight

C18H36O2 284.47 (for pure material) The USP32–NF27 describes stearic acid as a mixture of stearic acid (C18H36O2) and palmitic acid (C16H32O2). In the USP32– NF27, the content of stearic acid is not less than 40.0% and the sum of the two acids is not less than 90.0%.

Structural Formula



Stearic Acid

Functional Category

Emulsifying agent, solubilizing agent, tablet and capsule lubricant.

Applications in Pharmaceutical Formulation or Technology

Stearic acid is widely used in oral and topical pharmaceutical formulations. It is mainly used in oral formulations as a tablet and capsule lubricant; although it may also be used as a binder or in combination with shellac as a tablet coating. It has also been suggested that stearic acid may be used in enteric tablet coatings and

as a sustained-release drug carrier. In topical formulations, stearic acid is used as an emulsifying and solubilizing agent. When partially neutralized with alkalis or tri-ethanolamine, stearic acid is used in the preparation of creams. The partially neutralized stearic acid forms a creamy base when mixed with 5–15 times its own weight of aqueous liquid, the appearance and plasticity of the cream being determined by the proportion of alkali used. Stearic acid is used as the hardening agent in glycerin suppositories. Stearic acid is also widely used in cosmetics and food products.

Description

Stearic acid is a hard, white or faintly yellow-colored, somewhat glossy, crystalline solid or a white or yellowish white powder. It has a slight odor and taste suggesting tallow.

Typical Properties

Acid value	195–212
Boiling point	383°C
Density (bulk)	0.537 g/cm3
Density (tapped)	0.571 g/cm3
Density (true)	0.980 g/cm3
Flash point	113^{0}C (closed cup)
Melting point	69–70^{0}C
Moisture content	contains practically no water.

Partition coefficient Log (oil: water) = 8.2

Refractive index 1.43 at 80^0C

Saponification value 200–220

Solubility

It is freely soluble in benzene, carbon tetrachloride, chloroform, and ether. It's also soluble in ethanol (95%), hexane, and propylene glycol; practically insoluble in water.

Specific surface area

0.51–0.53m2/g [46, 47, 48]

EXPERIMENTAL METHODS

CALIBRATION CURVE FOR LOVASTATIN

PREPARATION OF CALIBRATION MEDIUM

27.218 gm of potassium-di-hydrogen phosphate was dissolved in sufficient amount of distilled water to make 1000ml of 0.2M solution. 8 gm of sodium hydroxide was dissolved in sufficient amount of distilled water to make one

1000ml of 0.2M solution. From the above solutions 50ml of potassium-di-hydrogen phosphate solution and 22.4 ml of sodium hydroxide solutions were mixed and made up to 200ml with distilled water to get phosphate buffer solution of pH 6.8.

Preparation of standard curve for valsartan

The standard stock solution of lovastatin was prepared by dissolving a known amount of drug in small quantity of phosphate buffer pH 6.8. From the above stock solution, different concentrations of 10, 20.....50λg /ml was prepared in same solution of phosphate buffer pH 6.8. The resulting solution was scanned in UV Spectrophotometer to find mmax and the absorbance was measured at λmax (239nm). The standard curve was plotted by taking concentration in X-axis and absorbance in Y-axis. The standard curve was used to estimate drug content and percentage drug release. [49, 50]

FORMULATION OF LOVASTATIN CONTAINING SOLID LIPID NANOPARTICLES BY HOT HOMOGENIZATION TECHNIQUE:

Drug-polymer compatibility study[51]

Drug-polymer compatibility study was carried out by FTIR (ATR). Spectra were recorded for pure drug, lipid and surfactant physical mixture. The spectrum recorded by grinding and dispersing the samples with micronized IR grade KBr powder.

Formulation of Lovastatin SLNs

The solid lipid nanoparticles of lovastatin were prepared by hot homogenization technique and there were nearly six formulations formulated with different concentrations of lipid, surfactant and stabilizer.

Solid Lipid Nanoparticles of Ibuprofen were produced using the lipid Stearic acid. The surfactant used for the formulation of SLN was Poloxamer 188 and stabilizer was Tween-80 in different proportions. The formulations were prepared by hot homogenization technique using the high speed homogenizer. The organic phase was prepared by dissolving the drug and surfactant in acetone: ethanol and mixing it with the melted stearic acid, Organic solvents were completely removed using a Buchi rotoevaporator. The drug-embedded lipid layer was melted by heating. The aqueous phase was prepared by mixing Tween-80 in water. The aqueous and organic phase was maintained at the same temperature. Then the organic phase was poured into aqueous Tween-80 solution. The various concentrations of Tween-80 which acts as a stabilizer and stirred with homogenizer on water bath at 10,000 rpm for 10 minutes by maintaining the temperature to 70^0c. The formulation was then removed from water bath and the dispersion of SLN was mixed gently by slow magnetic stirring for I hour at room temperature until cooling. [52, 53, 54] The formulation of SLN shown in table no: 1.

Table No: 1. Formulation of solid lipid nanoparticles of Lovastatin

Formulation Codes	Drug Lovastatin (mg)	Lipid Stearic acid (%w/v)	Surfactant Poloxamer 188 (%w/v)	Acetone: Ethanol (ml)	Water (ml)	Stabilizer Tween 80 (ml)
F1	40	0.5	0.5	10	100	0.5
F2	40	1.0	1.0	10	100	1.0
F3	40	1.5	1.5	10	100	1.5
F4	40	1.0	1.5	10	100	2.0
F5	40	1.0	1.5	10	100	1.5

Physicochemical properties

The solid lipid nanoparticles were characterized for physicochemical properties such as color, odor, pH, and taste.

Drug-polymer compatibility study

Drug-polymer compatibility study was carried out by FTIR (ATR). Spectra were recorded for pure drug, polymer and for drug and polymer physical mixture.

The FTI-IR spectrum pure drug sample and pure polymer sample with formulation were recorded to find out whether any interaction of drug with polymer was present in formulations.

Particle size and size distribution

Particle size analysis of the selected solid lipid nanoparticle formulation was performed using Malvern Mastersizer 2000.

Scanning Electron Microscopy (SEM)

The particle size of the Nanoparticles was evaluated by Scanning Electron Microscope (SEM) to find out the size of the nanoparticles and to study the surface morphology of the nanoparticles. Dried particles were taken in a piece of black tape and attached to the sample holder. Particle morphology was determined under low vacuum. The SEM method provides a finest approach to find out particles size and surface morphology when compared to other methods. [57,58]

Drug Entrapment Efficiency

The drug entrapment efficiency of the formulations was calculated by centrifugation of the aqueous suspension. The amount of the free drug was detected in the supernatant and the amount of incorporated drug was determined as the result of the initial drug minus the free drug. The percentage of incorporated drug called entrapment efficiency was determined spectrophotometrically at 238 nm. [10,59] The entrapment efficiency was calculated by using the following formula:

Entrapment efficiency EE (%) = $\frac{\text{Wt of initial drug – Wt of free drug}}{\text{Wt of initial drug}}$

In-vitro drug release study

The *in vitro* drug release was carried out by using diffusion studies by using magnetic stirrer. The release of Ibuprofen from the solid lipid nanoparticles was compared with the pure drug using a dialysis system comprising of a HiMedia Dialysis Membrane-70. The dialysis membrane was filled with 2 ml of solid lipid nanoparticles, and then the sample filled dialysis bags were tied and exposed to diffusion medium containing Phosphate buffer pH (6.8). The medium was stirred magnetically. The medium was maintained at a constant temperature of 37°C ± 0.5°C. Aliquots of 5ml samples were drawn at specified time intervals. The samples were analyzed by using a UV spectrophotometer at 238 nm. [10,60]

Stability Testing Studies

The stability study for the optimized formulation was performed for 3 months according to the ICH Guidelines. The SLN of Ibuprofen was kept at 30 °C ± 2 °C and 65% ± 5 % Relative humidity in stability chamber. Particle size measurement, Drug entrapment was evaluated. [61,62]

RESULTS AND DISCUSSION

CALIBRATION CURVE OF VALSARTAN

Calibration curve of lovastatin was done in phosphate buffer pH-6.8. Lovastatin shows λmax of 238 nm in phosphate buffer pH-6.8. The correlation coefficient was 0.999. Hence, lovastatin obeys the beer's law within the concentration range of 10 to 50µg/ml. The calibration plots of lovastatin in phosphate buffer pH-6.8 was showed in fig.2 and maximum absorbance showed in fig.3

FORMULATION OF LOVASTATIN CONTAINING SOLID LIPID NANOPARTICLES BY HOT HOMOGENIZATION TECHNIQUE:

Five formulations of lovastatin as solid lipid nanoparticles were prepared by using various concentrations of lipid, surfactant and stabilizer by using hot homogenization technique. The drug concentration kept as constant for each formulation (40mg).

The main reason for selection of the method was to improve the solubility of the poorly soluble drug by solid lipid nanoparticles and to improve the drug release pattern of lovastatin nanoparticles to show the sustained release of drug. The improved solubility and drug release leading to improvement of bioavailability of lovastatin.

EVALUATION OF FORMULATION

Drug-polymer compatibility study

FT-IR spectra of pure drug lovastatin, lipid and stabilizer and combination of physical mixture were evaluated. The figures are shown in Fig 4, Fig 5 and in Fig 6. From the obtained spectra it was observed that all the characteristics peaks of lovastatin were present in the combination spectra thus indicating the compatibility of the drug with the lipid used. It shows that there was no significant change in the chemical integrity of the drug.

Physicochemical properties

The solid lipid nanoparticles were characterized for physicochemical properties such as color, odor, pH, and taste.

Characterization of nanoparticles for particle size analysis

The particle size analysis of the nanoparticles reveals that the particle sizes were ranges from 80 – 250 nm and the particles were in nanometer size range. The SEM

Measurement of particle size and PDI

The particle size and PDI was found in range of 80.15 – 250.03 nm and 0.153 - 0.251 respectively for the SLN. The SLNs were found be within nanometer size range and it shows that increase in the concentration of both the surfactant and stabilizer leads to decrease in particle size whereas increase in lipid concentration leads to increase in particle size. The optimum concentration of surfactant and stabilizer was fixed as 1 % w/v. The Values were shown in Table.2, Fig 7 and in Fig 8.

Determination of percentage of drug entrapment efficiency

The drug entrapment efficiency was ranges from 75.76 % - 92.18 % of lovastatin SLNs and it shows the increase in concentration of lipid leads to increase in entrapment efficiency. The Values were shown in Table.2 and in Fig 9.

Determination of zeta potential

Zeta potential of the lovastatin SLNs nanoparticles was found in the range of -16.2±2.0 to -28.6±0.7mV. The results of Zeta potential revealed that all formulations were found stable. The Values were shown in Table.2.

Evaluation of In vitro **drug release**

The *In-vitro* drug release studies were carried out by using dialysis bag. The data of cumulative percentage drug release of the formulations were shown in Table.3, and in Fig 10. The cumulative percentage drug release after 8 h was studied

The percentage drug release lovastatin SLNs ranges from 52.20 – 80.62% at the end of 8 hrs. Among all the formulation the F3 which contains high concentration of surfactant and stabilizer (1.5%w/v) with optimum particle size shows 74.11% of drug release at the end of 8 hrs. The other two formulations with same concentration of surfactant and lipid (1.5% w/v) with higher concentration of

stabilizer also show increase in drug release. Based on the higher drug release compared to lovastatin plain solution and optimum particle size, the **F3** considered as best formulation for further evaluation. The comparison of *in vitro* release profile of F3 and lovastatin drug solution was shown in Fig 11.

Stability studies

Stability studies were carried out to find out the stability and changes in appearance and entrapment efficiency for optimized formulation F3. The formulation was stored at 30±2°C/65±5% RH for three months. The results were shown Table 4. The results showed that there was no significant difference in appearance and entrapment efficiency.

SUMMARY

- The purpose of this research was to develop novel solid lipid nanoparticles of lovastatin drug delivery system to improve the poor solubility and poor bioavailability of the drug.
- The novel solid lipid nanoparticles drug delivery system of lovastatin was prepared by using stearic acid as lipid, poloxamer 188 as surfactant and tween 80 as stabilizer by hot homogenization technique.

- Lovastatin used in the treatment of lowering cholesterol was successfully formulated in solid lipid nanoparticles.
- The formulated solid lipid nanoparticles of lovastatin was evaluated for physicochemical properties, Particle size and size distribution, Drug-polymer compatibility study, SEM, percentage of drug entrapment efficiency, zeta potential, *in vitro* drug release, Stability studies.
- The solid lipid nanoparticles were characterized for physicochemical properties such as color, odor, pH, and taste.
- The particle size analysis of the nanoparticles reveals that the particle sizes were ranges from 80 – 250 nm and the particles were in nanometer size range.
- The particle size and PDI was found in range of 80.15 – 250.03 nm and 0.153 - 0.251 respectively for the SLN.
- The drug entrapment efficiency was ranges from 75.76 % - 92.18 % of lovastatin SLNs and it shows the increase in concentration of lipid leads to increase in entrapment efficiency.
- Zeta potential of the lovastatin SLNs nanoparticles was found in the range of -16.2±2.0 to -28.6±0.7mV. The results of Zeta potential revealed that all formulations were found stable.

- The percentage drug release lovastatin SLNs ranges from 52.20 – 80.62% at the end of 8 hrs.
- Among all the formulation the F3 which contains high concentration of surfactant and stabilizer (1.5%w/v) with optimum particle size shows 74.11% of drug release at the end of 8 hrs.
- The other two formulations with same concentration of surfactant and lipid (1.5% w/v) with higher concentration of stabilizer also show increase in drug release.
- Based on the higher drug release compared to lovastatin plain solution and optimum particle size, the **F3** considered as best formulation for further evaluation.

- Stability studies were carried out to find out the stability and changes in appearance and entrapment efficiency for optimized formulation F3. The formulation was stored at 30±2°C/65±5% RH for three months.

CONCLUSION

- The results showed that there was no significant difference in appearance and entrapment efficiency.
- The FTIR results proved that there was no significant change in the chemical integrity of the drug and no interactions between the drug and polymers of lovastatin SLN.

- The methodology adopted for the improvement of poor solubility and poor bioavailability of the drug lovastatin solid lipid nanoparticles was effective.
- The lovastatin solid lipid nanoparticle system afforded sustained drug release over 8-h period of time.
- Lipid-based drug delivery systems like lovastatin solid lipid nanoparticles may contain a broad range of oils, surfactants, and co-solvents.
- They represent one of the most popular approaches to overcome the absorption barriers and to improve the bioavailability of poorly water-soluble drugs.
- The Solid lipid nanoparticle formulations can reduce the inherent limitation of slow and incomplete dissolution of poorly water-soluble drugs and facilitate formation of solubilized phases from which absorption may occur.
- The co-administration of lipids with drugs can also impact their absorption pathway although most orally administered compounds gain access to the systemic circulation via the portal vein, some highly lipophilic drugs are transported directly to the systemic circulation via intestinal lymphatic's, which improves oral bioavailability of poorly water soluble API like lovastatin.
- The solid lipid nanoparticles of lovastatin formulation can be effective in treatment of lowering cholesterol and the drug was released from the formulation in a constant manner for the desired period of time.

- The solid lipid nanoparticles of lovastatin evaluated here has potential in reduction of cholesterol, for the reason that improved solubility can improve the bioavailability of the poorly soluble drug lovastatin and considered to be promising for prolonging the release of drug compared with other conventional formulations.

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